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OBSERVATIONS ON THE NATURE OF THE BENCE JONES ALBUMIN.

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The clinical history of the patient, whose urine I was enabled to study through the courtesy of the attending physician, Dr. N. E. B. Iglehart, of Baltimore, will be reported in greater detail at another place by Dr. L. P. Hamburger. A preliminary report has appeared in the *Johns Hopkins Hospital Bulletin*[1] of February, 1901. From this I have extracted the following brief account of the patient's illness, which is supplemented by a communication from Dr. Iglehart on the subsequent course of the disease.

The patient, a lady, aged forty-nine years, whose previous health had been good, was suddenly seized with sharp pain over the ninth left rib near its cartilaginous attachment (August, 1900). The pain was severe, and increased on deep inspiration. There was tenderness on pressure over the painful point. The condition at the time so nearly resembled a fracture that it was supposed the patient had injured the rib. A history of trauma, however, could not be obtained, and there was neither crepitus nor a friction rub. Within three weeks the pain had disappeared. She was again seen in September, and at that time complained of nausea, without apparent cause. She had lost thirteen pounds in weight, and her general health had manifestly suffered. Early in October she was again seized with pain, but this time in the region of the eighth rib, in the mid-axillary line. The patient then remarked that since the past summer she had taken more water and had voided a larger quantity of urine than usual (on one occasion 3500 c.c. were passed in twenty-four hours).

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[1] L. P. Hamburger. Two Examples of Bence Jones Albumosuria Associated with Multiple Myeloma.

Dr. Osler saw the patient on November 30th, when, aside from a slight pallor of the visible mucous membranes, the physical examination was negative. In February of the following year a slight but definite elevation was palpable over the eighth right rib in front, of the size of a lima bean. The points referred to continued to be tender to pressure throughout the life of the patient. Subsequently she suffered a great deal of pain, the attacks becoming more and more frequent, but separated even then by periods of comparative comfort. Especially in the upper part of the left thigh there was much pain, which began about three months before death. For a while she used a cane; later she was obliged to resort to crutches, and finally she could not walk at all, and had to take to a wheel-chair. There was no deformity of the bone apparent, and palpation revealed no thickening of the femur, but it was very painful. There was marked muscular atrophy. The general loss of flesh increased, and toward the end amounted to about forty pounds. Nausea and vomiting became more and more marked, and were finally uncontrollable. Even small amounts of water could not be retained. The anæmia also increased markedly, but at no time suggested a pernicious type, so far as the general appearance of the patient went. An examination of the blood, however, was not made. The polyuria which was observed earlier in the disease continued to the last three weeks, but was not so marked as at first. During the last ten days the urine was scant, but the amount had not been estimated. Five days before death spontaneous fracture of the left femur occurred in its upper third, and with gradually increasing weakness death occurred on August 16, 1901. A complete autopsy, unfortunately, could not be made. Examination of the sternal end of what was regarded as the eighth right rib, however, very curiously, showed no abnormality.

The diagnosis of multiple myeloma was made at the time of the second attack of pain, on the basis of a urinary examination. Of this Dr. Hamburger writes as follows: A specimen taken from the collected amount of twenty-four hours (3500 c.c.) was very pale, of acid reaction, with a specific gravity of 1004. It gives a white ring when floated over nitric acid. Heated to a temperature of about 55° C. a heavy, milky-white precipitate appeared. On boiling the fluid becomes clearer to become more turbid again on cooling. The addition of acetic acid to the fluid after reaching its maximum turbidity causes it to clear again. A few drops of nitric acid yield a precipitate which dissolves completely on boiling and reappears on cooling. In the Esbach albuminimeter the proteid content reached 0.27 per cent. The urine gives a strong biuret reaction. No casts were seen in a centrifugalized specimen. From these reactions Dr. Hamburger concluded that the urine contained the so-called albumose of Bence Jones, which, according to Kahler, is only found in cases of myeloma. This diagnosis was accordingly made. With Dr. Hamburger's consent I then undertook a more detailed study of the substance in question, with which the present paper has to deal. Through the courtesy of Dr. Iglehart about 16,000 c.c. of the urine were placed at my disposal, which was partly examined at once, partly after preservation with thymol or chloroform.

The urine was sent to me in bulk, without any attempt at separate collection, for periods of twenty-four hours, so that I am unable to give accurate figures in this respect from personal knowledge. Dr. Iglehart, however, informs me that during the period of polyuria, which was first noted about August 10, 1900, and which extended over nearly a year, the average elimination varied between 2000 and 3500 c.c.

General Properties of the Urine. The urine was very pale and markedly cloudy. Microscopical examination showed that the turbidity was referable to numerous epithelial cells, derived from the genitals, and a fairly large number of leucocytes, probably of the same origin. There were no crystalline elements, no red blood-corpuscles, and no casts. Even after standing for five months a crystalline deposit of the albuminous body was not observed, nor did the substance separate out in amorphous form.

As in Magnus-Levy's case, it was noted that the urine underwent ammoniacal decomposition much less readily than is common. The reaction was constantly acid; the specific gravity usually low, never exceeding 1015.

A study of the common constituents of the urine was not made, but it was noted that uric acid and indican were not increased and that sugar was absent. Special attention was directed to the behavior of the peculiar albuminous body, which was present in considerable amount. Quantitative estimation (gravimetrically) on two occasions gave 0.5072 and 0.5124 per cent. These higher figures, however, were only found in the first lots of urine, while later on the amount was much smaller, and an examination of a 5-litre specimen which had been kept over the summer showed the presence of only about 0.025 per cent.

The general reactions which were obtained with the native urine are the following:

1. Upon the application of heat the acid urine remains clear until a temperature of about 52° C. is reached, when the liquid begins to show a milky turbidity. This rapidly increases in intensity, and at 55° C. the entire volume of urine is perfectly opaque, so that the bulb of the thermometer can no longer be seen in the test-tube. When heated still higher the milky turbidity is replaced by the appearance of flocculi, but even as the boiling-point is reached the urine can scarcely be said to have become clearer. On subsequent cooling the flocculent precipitate is seen at the bottom of the tube as a peculiar, granular-looking sediment, and its amount does not suggest an increased precipitation during cooling.

2. Upon the addition of a drop or two of a 25 per cent. solution of nitric acid to a few c.c. of urine a slight cloud forms, which disappears on shaking. When more of the acid is added an abundant precipitate results, which is insoluble in a large excess of acid (ten times the original volume). On heating the acid solution the albuminous substance first coagulates to a dense mass, but dissolves almost entirely on prolonged boiling, with the formation of a pink foam. On subsequent cooling the precipitate reappears, to disappear again (almost entirely) on boiling.

It is noteworthy that *with the cold nitric acid test, applied in a conical glass, a far more abundant precipitation is manifestly obtained than upon the application of heat.*

3. With hydrochloric acid and sulphuric acid similar results are obtained. As a rule, three drops of the concentrated acid (as also of nitric acid) were necessary to bring about a precipitation of the albumin, which did not disappear on shaking.

4. Carbonic acid was without effect, even if the urine had been copiously diluted.

5. Upon the addition of acetic acid, even in large excess, no precipitate results. On subsequent heating the liquid remains clear.

6. Phosphoric acid also does not cause a precipitation of the substance.

7. The addition of picric acid (13 to 14 drops of a saturated solution for 5 c.c. of urine) causes the formation of a flocculent precipitate, which increases in amount on heating, but does not disappear entirely on boiling. With the mineral acids complete clearing was not always observed, but in this case the urine remained much more turbid.

8. Tannic acid causes complete precipitation after the previous addition of acetic acid. The precipitate disappears to a great extent on boiling.

9. With phosphotungstic acid, in the presence of hydrochloric acid a heavy precipitate results, which does not disappear on boiling.

10. With potassium ferrocyanide and acetic acid a precipitate is obtained which is but little soluble at 100° C.; it increases on subsequent cooling.

11. On neutralization with a dilute solution of sodium hydrate or ammonia no precipitation results.

12. On acidifying the urine with acetic acid and subsequent neutralization with sodium hydrate a precipitate results, which dissolves practically entirely on boiling and reappears on cooling.

If an excess of sodium hydrate is added to the neutral solution the neutralization precipitate disappears, and it is noteworthy that no precipitate now appears on heating; but if then the solution is again neutralized with acetic acid a neutralization precipitate appears; but, in contradistinction to the first, this does not disappear on boiling, and is only slightly soluble in the presence of a large excess of the acid.

13. If the urine is first rendered alkaline with sodium hydrate and is then neutralized with acetic acid, a neutralization precipitate results, which, as in the last instance, does not disappear on boiling, and is only slightly soluble in a large excess of the acid.

In order to obtain such neutralization precipitates, however, not too little acid or alkali must be added, as otherwise no precipitate occurs on subsequent neutralization. In one instance I treated 20 c.c. of a solution of the isolated substance (precipitation with ammonium sulphate and dialysis for eight weeks) with a few drops of hydrochloric acid—such that the solution contained 0.36 per cent. On neutralization with sodium hydrate a precipitate was then not obtained, even on heating; but this developed at once on acidifying the hot solution with acetic acid.

14. Similar results as in 11 and 12 were obtained with dilute mineral acids.

15. Alcohol, when added in the proportion of two volumes for one of the urine, causes complete precipitation of the substance; and it is to be noted that after prolonged contact the albumin loses its subsequent solubility in water.

16. On treating the urine with an excess of a saturated solution of common salt (two to three times the original volume) no change occurs, but on heating the substance is precipitated, and on subsequent boiling the solution remains turbid.

17. Upon the addition of a saturated solution of common salt to the acidified urine (acetic acid) complete precipitation occurs, which is hastened by heating to about 50° C. On boiling there was no evidence of clearing.

18. Saturation of the urine with rock salt at ordinary temperatures causes a partial precipitation, which is soluble in water. Saturation at from 35° C. to 40° C., on the other hand, results in the complete precipitation of the albumin, and it is noteworthy that this precipitate is insoluble in water.

19. Upon the addition of two and one-half volume of a saturated solution of magnesium sulphate to the urine no change occurs, even after the addition of acetic acid; but on saturating the acidified solution with the salt in substance the albumin is precipitated. This occurs at once if the urine is first saturated with the salt, and acetic acid is then added. *Saturation of the urine with magnesium sulphate without the addition of acetic acid caused no change. In a dialyzed specimen, however, which presented a neutral reaction complete precipitation could be produced by saturation with the salt in question.* (See below.)

20. Saturation of the acidified urine with sodium sulphate in substance causes the precipitation of the albumin, as in the case of magnesium sulphate. The addition of an equal volume of a saturated solution of the salt causes partial precipitation.

21. A saturated solution of ammonium sulphate, when added in the proportion of two volumes for one volume of urine, causes the complete precipitation of the substance, the reaction being neutral.

22. On dialysis the substance is not precipitated, nor does it pass through the membrane.

23. Millon's reaction: On heating the flocculent precipitate turns red.

24. Biuret reaction: An intense purplish-violet is obtained if a sufficient amount of the copper solution is added; if but little is present the color is red.

25. Reaction of Adamkiewicz: A beautiful purplish-violet results.

26. Hydrochloric acid reaction: A reddish-violet color results, but is not nearly so intense as with Adamkiewicz's test.

27. The sulphur test yields a positive result (brown color on boiling).

28. The xanthroproteic reaction is positive.

29. Molisch's reaction is not well marked, but occurs on prolonged standing.

Reactions of the Isolated Substance.[1] To isolate the Bence Jones substance the urine was neutralized and precipitated with twice its volume of a saturated solution of ammonium sulphate. The copious precipitate was filtered off with the aid of a suction pump, and kept as such in a two-thirds saturated solution of the salt. When first precipitated it appeared white; but later, after exposure to the air, it assumed a pinkish tint, which subsequently changed to a light terra-cotta.

To purify the substance the material was repeatedly dissolved in hot water, reprecipitated with ammonium sulphate, and finally dialyzed. It was noted, however, that it was impossible to remove all the sulphate in this manner, even after exposure to running water for nearly three months. The resulting solution was used for the common tests, while it was found necessary to resort to some other method of purifying the substance in order to obtain material that could be employed for purposes of elementary analysis. To this end the filtered urine was slowly heated and the albumin thus coagulated. The coagulum was washed with water, dissolved by the aid of a little sodium carbonate in boiling water, and repeatedly precipitated with alcohol. The precipitated substance was finally placed in the dialyzer for twenty-four hours and the contents of the dialyzer treated with double their volume of alcohol, so as to recover a portion of the substance that had passed into solution. The material was washed with absolute alcohol and ether, pressed out between filter paper, and dried at 105° C., when it changed to a horny, brittle mass. Purified in this manner the substance was practically free from ash, while the crude material apparently contained both iron and phosphorus. Two nitrogen estimations gave 15.12 and 15.26 per cent.

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[1] Through the kindness of Dr. Edward S. Wood, of Harvard, I was enabled to compare the reactions of my isolated substance with a product which he obtained from the urine of a patient of Dr. Fitz (Case 13, see below). The corresponding report follows below.

The dried substance is almost insoluble in water and dilute saline solutions at ordinary temperatures, and dissolves with difficulty even in hot water unless a trace of sodium carbonate is added. In that event, however, solution takes place with comparative readiness on heating, and it is to be noted that the solution remains clear on subsequent neutralization, providing that a sufficient amount of water is present and that too much of the soda has not been added. The *moist* substance, on the other hand, is soluble even in cold distilled water, though not so readily as the references in the literature would lead one to think. It is noteworthy that it is not nearly so easily soluble as the common water-soluble digestive albumoses. On prolonged contact with alcohol the material is rendered insoluble in water and dilute saline solutions; in such an event, however, as has already been pointed out, solution in water can be effected on heating after the addition of a little soda or a few drops of hydrochloric acid. Such a precipitate, moreover, is soluble in dilute ammonia.

The solution of my isolated substance (obtained, as first described, by precipitation with ammonium sulphate and subsequent dialysis) was of neutral reaction, and on heating became but slightly turbid. If a small amount of salt and a drop of acetic acid were added, however, coagulation occurred as in the case of the native urine. Heated *slowly*, immersed in a beaker with water, the first trace of turbidity was noticeable at 54.5° C.; at 55° C. the entire bulk of the solution was cloudy; at 55.5° C. it was decidedly opaque; the maximum intensity was reached between 58° C. and 60° C. At about 70° C. the general opacity was replaced by the appearance of a flocculent precipitate, which gradually assumed a more granular appearance and finally settled to the bottom in the form of microscopical spheroliths, while the supernatant fluid became clear. An actual solution of the substance was not observed.

On heating the solution and then adding concentrated nitric acid a flocculent precipitate occurred with the simultaneous appearance of the xanthoproteic reaction.

Upon the addition of nitric acid to the cold solution a precipitate occurred, which, in contradistinction to the native urine, was but little soluble on the subsequent application of heat, even at 100° C., and on cooling no appreciable increase in turbidity was noted. The same result was obtained with potassium ferrocyanide and acetic acid.

On treating the solution with an acid or an alkali and subsequent neutralization a precipitate occurred, which was soluble in an excess of the reagent. To obtain this precipitate, however, it was necessary to add a definite amount of acid or alkali; if too little was used no precipitate occurred. This is shown by the following example: 20 c.c. of the clear neutral solution were treated with an amount of hydrochloric acid such that the solution contained 0.36 per cent. On neutralization with sodium hydrate no precipitate occurred, and on boiling the solution remained clear. If, then, a little acetic acid was added, however, while the solution was still hot, precipitation occurred at once.

On saturating the *neutral* solution with magnesium sulphate at the temperature of the room, complete precipitation occurred on standing for less than twenty-four hours.

The limits of precipitation with ammonium sulphate were 2.3 and 3.8, respectively.

On salting with common salt to saturation a partial precipitation of the substance occurred at ordinary temperatures, the reaction being neutral. On adding two to three times the volume of a saturated solution of common salt to a solution of the isolated substance, however, no precipitation occurred so long as the reaction was neutral, but on adding a few drops of acetic acid a turbidity occurred at once.

On salting with rock salt at ordinary temperatures an abundant precipitate also occurred, which, like the one preceding, was soluble in water.

Upon the addition of a dilute solution of copper sulphate precipitation occurred at once. The biuret reaction was positive—an intense purplish-violet.

In its other reactions the isolated substance showed the same general behavior as the native urine (which see).

From these reactions of the urine and the isolated substance it is clear that the albuminous body in question is not one of the common albumins which we meet with in ordinary cases of albuminuria, but clearly resembles the substance which was first observed by Bence Jones and MacIntyre in a case of supposed osteomalacia.

Special Reactions. Behavior of the Substance on the Application of Heat. Of especial interest is the behavior of the urine on the application of heat. Quite constantly the first indication of a turbidity appeared as the 52° C. mark had just been passed and before 53° C. had been reached. At 55° C. the liquid had become quite opaque, so that it was impossible to see the markings of the thermometer where it was immersed, and still later even obscuring the bulb of the instrument. The turbidity was distinctly milky in appearance, and was first noted in the surface layer, from which it gradually extended downward. Later the starting-point of the precipitation appeared to be wherever the urine came in contact with the walls of the test-tube or the bulb of the thermometer. Heated beyond 55° C., viz., to 60° C., the turbidity sometimes increased a little more, providing that the elevation of the temperature was brought about gradually, as by immersing the tube in a large beaker full of water and heating this with a comparatively small flame. On further heating no increase in opacity was noted, but as 75° C. to 80° C. was reached the milky turbidity gave way to the appearance of a coarsely flocculent precipitate, which, however, did not dissolve even at 100° C. If left to itself the precipitate settles to the bottom, while the supernatant fluid becomes clear, and remains clear on gradual cooling. If, however, the liquid is suddenly cooled, as by immersion in cold water, the supernatant fluid becomes turbid. A similar result is reached on filtering the hot liquid, when the first portion which passes through at once turns cloudy on coming in contact with the cold walls of the tube or beaker.

Providing that the process of heating is carried on very gradually and the urine is then allowed to cool of itself, a solution is obtained on filtration which no longer becomes turbid on subsequent heating at any temperature, but in which an albuminous substance is manifestly still present. Barring its different behavior toward heat, this solution gives the same general reactions as the native urine, viz.:

1. On treating with potassium ferrocyanide and acetic acid the solution becomes turbid, and on heating the turbidity does not disappear, but becomes even more pronounced. At 100° C. it is but slightly soluble.
2. With nitric acid a marked reaction is obtained.
3. After the addition of acetic acid, even in excess, the solution remains clear.
4. A marked biuret reaction is obtained—a purplish-violet, as in the native urine.
5. Treated with an equal volume of a saturated solution of common salt, no change occurs; but upon the addition of a few drops of acetic acid a turbidity results, which increases upon the application of moderate heat, but disappears on boiling, to reappear on cooling, etc.
6. Treated with two volumes of a saturated solution of ammonium sulphate the albuminous substance is completely precipitated.

The peculiar behavior of the urine on heating, on first consideration, suggested the presence of two distinct albuminous bodies in the urine, one of which could be coagulated by heat, while the other was apparently non-coagulable. It was ascertained, however, that such was not the case, and that it is possible by a suitable variation of the reaction, and by increasing or diminishing the amount of salt present, to increase or to diminish at will the relative amount of the coagulable as compared with the apparently non-coagulable portion. With an alkaline specimen it was thus found that after heating a much larger amount of the albumin remained in the filtrate than in the case of the acid urine, and as I have already indicated it is possible to completely precipitate the total amount of the substance by heating, after the previous addition of an equal volume of a saturated solution of sodium chloride to the acidified urine (acetic acid). In such an event the resulting filtrate is free from albumin.

It was also noted that the rapidity of heating is of marked influence upon the extent to which coagulation occurs. The more gradually this is done the more extensive is the coagulation. That the temperature at which this occurs is also dependent, to a certain extent at least, upon the amount of salt present was first shown by Magnus-Levy, and was also noted in the present instance. It was thus ascertained that whereas coagulation occurred in the native urine at a temperature of from 52.5° C. to 54° C., it was necessary to heat to 55° C. or 56° C. if a double volume of a saturated solution of sodium chloride had been added. The further addition of salt then lowers the temperature of coagulation, and on saturating the urine with salt in substance complete coagulation may occur at 37° C. already if the liquid is kept at this temperature for several hours.

This influence of the relative amount of salt upon the degree of heat at which coagulation occurs is well shown in the following table, which is taken from Magnus-Levy:

Urine.	*20 per ct. salt sol.*	*Water.*	*First turbidity at*
2 c.c.	0.0 c.c.	2.0 c.c.	54.0°
2 "	0.2 "	1.8 "	54.5
2 "	0.4 "	1.6 "	55.0
2 "	0.8 "	1.2 "	55.0
2 "	1.2 "	0.8 "	55.5
2 "	1.6 "	0.4 "	56.0
1 "	3.0 "	0.0 "	56.5

Ammonium chloride, in my experience, did not influence the temperature of coagulation, while it apparently renders the precipitated substance more readily soluble at 100° C. This, however, is only observed if a definite amount of the salt is present, which should not exceed 1 to 2 per cent. saturation. At times, moreover, no result at all was obtained without any apparent reason. On one occasion two specimens of urine were diluted, the one with 2 c.c. of a saturated solution of ammonium chloride and the other with water. On heating neither became especially cloudy (the heat was rapidly applied). On cooling the slight turbidity persisted and even increased to a slight extent. On subsequent heating the ammonium chloride specimen now became more turbid than the control, and did not clear whatever on boiling. Upon the addition of a little acetic acid the turbidity increased even more.

Of great interest in this connection is the observation of Spiro[1] that the addition of urea to solutions of albumins increases the degree of temperature at which coagulation occurs, and that a point may be reached at which no coagulation whatever takes place. In accordance with Hofmeister's suggestion, that this might possibly account for the varying statements as regards the solubility of the precipitated albumin of Bence Jones at 100° C., Magnus-Levy undertook a special examination of his substance in this direction. His results are quite in accord with those reached by Spiro in the case of egg albumin and serum albumin. I have repeated this work, and was able to show that, as a matter of fact, a point is reached, on the gradual addition of a urea solution in increasing amounts, at which coagulation no longer occurs; but I was not able to demonstrate that the temperature of coagulation was correspondingly raised. So long as a turbidity occurred at all the temperature was the same until a certain percentage was reached, when the solution remained clear altogether. These results are shown in the following tables:

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[1] K. Spiro. Ueber die Beeinflussung der Eiweisscoagulation durch stickstoffhaltige Substanzen, Zeit. f. physiol Chem., 1900, vol. xxx. p. 182. See also the recent work of W. Pauli and P. Rona, Untersuchungen über physikalische Zustandsänderungen d. Kolloide, Beiträge z. chem. Phys. u. Pathol., 1902, vol. ii. p. 1.

EFFECT OF UREA UPON THE COAGULATION OF BENCE JONES ALBUMIN

Urine (neutral).	Urea solution (50 per ct.).	Water.	Per cent. of urea added.	On warming.	At 100° C.	On cooling.	Repeated warming and cooling.
2 c.c.	0.0 c.c.	2.0 c.c.	....	Marked turbidity.	Slight clearing.	Slight increase.	The same.
2 "	0.2 "	1.8 "	2.5 per ct.	Marked turbidity.	Marked clearing.	About the same.	The same.
2 "	0.4 "	1.6 "	5.0 "	Momentary cloud.	Clear.	Clear.	Clear.
2 "	0.5 "	1.5 "	6.25 "	Clear.	"	"	"
2 "	0.6 "	1.4 "	7.5 "	"	"	"	"
2 "	0.7 "	1.3 "	8.7 "	"	"	"	"
2 "	0.8 "	1.2 "	10.0 "	"	"	"	"
2 "	1.0 "	1.0 "	12.5 "	"	"	"	"

Magnus-Levy states that by diluting the clear solution, containing an excess of urea, with water, the albumin is precipitated even at ordinary temperatures. This observation I was unable to verify in my case. After the addition of two to three times the amount of water, at least, no turbidity was noted, even on the application of heat.

In attempting to ascertain the effect of urea upon the coagulation point it was only possible to note the first appearance of a turbidity, about the maximum turbidity, about the beginning of clearing, and roughly the point of maximum clearing. A flocculent precipitate was not obtained in these experiments.

Urine (neutral).	Water.	Urea solution (50 per ct.).	First turbidity.	Maximum turbidity.	First clearing.	Maximum clearing.
2 c.c.	2 c.c.	0.0 c.c.	53.5°	60.0°	79.0°	92.0°
2 "	1.8 "	0.2 "	53.0	60.0	75.0	92.0
2 "	1.6 "	0.4 "	53.0 (?)	Clear.	Clear.	Clear.

Like Magnus-Levy, I observed that the addition of a moderate excess of urea will dissolve the precipitate which has been produced by heat, even at ordinary temperatures, though at times this did not occur, for reasons which were not apparent.

Limits of Precipitation with Ammonium Sulphate. For purposes of comparison with other albuminous substances, the limits of precipitation with ammonium sulphate were determined both in the native urine and in a dialyzed specimen of the isolated substance according to Hofmeister's method, as described by Pick.[1] To this end a series of test-tubes was prepared, such that each contained 2 c.c. of the urine (viz., the dialyzed specimen) and an increasing amount of a saturated solution of ammonium sulphate, the mixture in each case being diluted with distilled water to 10 c.c. The concentration at which the first permanent turbidity occurred was regarded as the lower limit. The mixture in every case was thoroughly agitated, usually allowed to stand for one-half hour and then passed through a double filter; but at times it was necessary to wait for several hours (sometimes for twenty-four hours). To the clear filtrate of each tube 0.2 c.c. of the salt solution was then further added, in order to ascertain whether all of the substance had been precipitated. The concentration of the first specimen in which a further turbidity no longer occurred indicated the upper limit of precipitation. The results which were obtained in the case of the native urine were 0.7 and 4.2, and with a solution of the isolated substance 2.3 and 3.8, respectively.

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[1] E. Pick. Untersuchungen über die Proteinstoffe, Zeit. f. phys. Chem., vol. xxiv. p. 246.

Behavior on Digestion with Pepsin-hydrochloric Acid. To test the behavior of the albumin on peptic digestion a series of experiments was undertaken. The substance was previously isolated either by precipitation with ammonium sulphate or with alcohol, and purified as described. In some cases the solid substance was used, in others a solution. The degree of acidity of the digestive mixture corresponded to from 0.3 to 0.4 per cent. of hydrochloric acid. The pepsin used was a preparation of Merck's. Examination showed that this was suitable for our purposes. It was clearly soluble in water, gave only a faint biuret reaction, and, treated with an equal volume of a saturated solution of ammonium sulphate, a very faint turbidity. The digestive mixture was kept at a temperature of from 35° C. to 40° C. for a varying length of time. In the first experiment digestion was allowed to continue for a week. At the expiration of this time a thin deposit of an amorphous substance had appeared, of the nature of which nothing could be ascertained. The liquid was filtered and the filtrate neutralized. A precipitate did not occur. The solution was then acidified with acetic acid and treated with an equal volume of a saturated solution of sodium chloride and boiled. The clear filtrate was then treated with an equal volume of a saturated solution of ammonium sulphate, but after standing for nearly seventy-two hours a precipitate had not formed. There was merely a slight turbidity, which could not be removed by filtration. The solution was now further treated with the ammonium sulphate solution to the extent of one-half of the total volume, but again no precipitate resulted, even on standing four times twenty-four hours. The liquid had become somewhat more turbid, but could not be cleared on filtration. It was then saturated with the salt in substance. On standing a gummy material separated out, which manifestly represented the deutero-albumose B of Pick. The substance gave an intense purplish-red biuret reaction and a positive sulphur reaction (at first a brown color, later a precipitate of black sulphide of lead). The reaction of Adamkiewicz was positive, while Molisch's reaction was negative. Dilute copper sulphate solution caused no precipitation. On digestion with trypsin much tyrosin and comparatively little leucin were obtained.

After separation of the deutero-albumose B the clear filtrate was treated with one-tenth its volume of a very dilute solution of sulphuric acid saturated with ammonium sulphate—such that the reaction was feebly but distinctly acid. On standing even for several days a precipitate could not be obtained; only a slight turbidity resulted, which could not be removed by filtration. Deutero-albumose C, if present at all, was hence only formed in traces. On treating the filtered solution with Lugol's solution saturated with ammonium sulphate, a precipitate formed, which on standing appeared as a gummy, black mass that closely adhered to the walls of the vessel. This was soluble in alcohol, and represents Pick's peptone B. A substance corresponding to Pick's peptone A was present, if at all, only in traces.

In the above experiments no evidence of the formation of primary albumoses was obtained; and as Magnus-Levy claims to have isolated a proto-albumose among the products of peptic digestion in his case, I repeated the experiment, allowing the digestion to continue for only twenty-four hours. The preparation used for this purpose was obtained by precipitation with alcohol, and was placed in the digestive mixture in the solid state. After twenty-four hours a small amount of an amorphous sediment was observed, as in the first instance, but it was likewise insufficient in amount for purposes of special examination. On neutralization no precipitate appeared. On the contrary, the liquid, which was distinctly turbid even after filtration, became almost clear as the neutral point was reached. It was then acidified by the addition of a couple of drops of acetic acid, and it was noted that on the addition of each drop a slight turbidity resulted, which disappeared on shaking. The solution was then treated with an equal volume of a saturated solution of sodium chloride. Upon the application of heat a beginning turbidity was noted between 52° C. and 53° C. This reached its height between 58° C. and 60° C., and was replaced between 78° C. and 80° C. by a cloudy, flocculent precipitate which became finely flocculent on boiling, but did not disappear. On cooling, what appeared to be an undigested portion of the Bence Jones albumin was filtered off, the filtrate neutralized, and further treated as described above. In this case, also, deutero-albumose B was obtained in considerable amount, while both deutero-albumose A and C were present only in traces.

As in this case, also, primary albumoses were not obtained, a third series of experiments was undertaken in which digestion was interrupted after from six to ten hours. About 10 grammes of the carefully purified substance were used and placed in the digestive mixture *en masse*. The process of "digestion" was closely followed and interrupted as soon as all the material had passed into solution. My special aim in these experiments was to separate from each other the undigested portion of the Bence Jones substance and any primary albumoses that may have been formed. To this end I first proceeded as described, viz., the solution was neutralized (no precipitation occurred) and then concentrated to a smaller volume. As in my case, the limits of precipitation of the substance were such that a satisfactory separation of the original substance from primary albumoses by fractional precipitation with ammonium sulphate was not possible, as in Magnus-Levy's case, I was forced to resort to some other plan. After a number of trials it was found that this separation could be readily effected by alcohol. It has been found that the Bence Jones substance could be completely precipitated by adding two volumes of alcohol (95 per cent.), and Pick[1] has shown that proto-albumose is soluble in alcohol of less than·80 per cent., while hetero-albumose is thrown down already in the presence of from 25 to 32 per cent. I accordingly precipitated the neutral digestive solution with an equal volume of a saturated solution of ammonium sulphate and allowed the mixture to stand for forty-eight hours. The fairly abundant precipitate which had collected by that time was then filtered off, washed with a small amount of iced water, stirred up with water at an ordinary temperature, and the resulting solution treated with twice its volume of 94 to 95 per cent. alcohol. The mixture was then allowed to stand for at least twenty-four hours. Any undigested portion of the Bence Jones substance—and I must confess that I did not find that it was digested so very rapidly as the statements in the literature led me to believe, although it *dissolved* comparatively rapidly—was thus thrown down and could readily be filtered off. The clear alcoholic filtrate was freed from alcohol by distillation in the vacuum. There remained a *clear* solution, which gave all the reactions of proto-albumose, and which could scarcely contain any other substance. A hetero-albumose was certainly not present in this fraction. Its absence in the alcoholic precipitate also was proved in another series of experiments by fractional precipitation with alcohol. On boiling the filtered alcoholic solution, moreover, the occurrence of a turbidity was never observed if care had been taken to allow the solution to stand sufficiently long so that everything that could be precipitated by alcohol in the cold had separated out.

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[1] E. P. Pick. Zur Kenntniss d. peptischen Spaltungsprodukte d. Fibrins, Pt. I., Ztlsch. f. physiol. Chem., 1899, vol. xxviii. p. 219.

In this manner, then, *the formation of proto-albumose from the Bence Jones substance could be satisfactorily demonstrated.* The amount of proto-albumose which was obtained was never large, but manifestly much more abundant than the amount of fraction I which Umber[1] could isolate among the digestive products of serum-albumin, of which he writes that it was always present in such small amounts, that it only appears as a faint opalescence on treating the digestive mixture with an equal volume of the sulphate solution, no matter whether the attempt was made to isolate it after three or not until after eighteen hours. He adds: "It would thus seem as though the fraction by no means represented a transitory stage for all following albumoses, which are present in abundance, unless, indeed, it is further decomposed already in *status nascendi.*" This observation I regard as important, in view of the fact that, as I shall show later on, the Bence Jones substance is in all probability a globulin, or at least closely related to the class.

After precipitation of the proto-albumose, together with the undigested portion of the original substance, by ammonium sulphate and their separation by filtration, the filtrate was then further treated as described, and with the same results. Deutero-albumose B and peptone B were always obtained in a fairly large amount. Deutero-albumose A also could be isolated in amounts sufficient to establish its identity; but of deutero-albumose C and peptone B only traces could be found, if, indeed, peptone B was present at all.

Crystallization. I have made a number of attempts to obtain the substance in crystalline form, but have thus far not been successful. I have noted, however, like others, that the granular-looking sediment which collects at the bottom of the vessel when the substance has been slowly precipitated by heating is composed of typical spheroliths, such as Hofmeister describes.

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[1] F. Umber. Die Spaltung d. krystallinischen Eier und Serumalbumins sowie d. Serumglobulins durch Pepsinverdauung. Zeitsch. f. physiol. Chem., 1898, vol. xxv. p. 258.

While I was about to finish my work on the present case, and was surveying the literature on "Bence Jones' albumosuria," Dr. Edward S. Wood, of Harvard University, very kindly presented me with about 50 grammes of the isolated substance, for comparison, which he had obtained from the urine of Dr. Fitz's second case, and which was reported by Wright (see below). In this case, also, the diagnosis of multiple myeloma was made during the life of the patient and confirmed by autopsy. Dr. Wood wrote me that the substance had precipitated at a temperature between 50° C. and 60° C., but dissolved by heating to boiling, and precipitated again on cooling. It contained 0.2 per cent. of phosphorus, and owing to this observation, no doubt, Dr. Wood regarded the substance as a nucleo-albumose. Elementary analysis gave the following results, which, together with those obtained by other observers, I have placed in the accompanying table:

	C	H	N	O	S	P
Bence Jones	52.04	7.08	15.02	....	1.09	0.19
Kühne	52.13	6.83	16.0			
Neumeister	52.87	6.91	15.55	....	1.12	0.0
Magnus-Levy	....	....	15.56	....	....	0.0
Wood	42.3	7.72	13.85	35.76	0.16	0.21

The product which Dr. Wood used for elementary analysis was prepared by precipitating the urine with 95 per cent. alcohol, filtering, and reprecipitating with alcohol. This process was repeated at least three times, the alcohol being allowed to act upon the precipitate for several days at each time. The final purified product was dried over sulphuric acid in a vacuum to a constant weight. Comparison with the figures obtained by others suggests that even so the product was not quite pure, and I have succeeded, as a matter of fact, in obtaining it free from phosphorus by appropriate treatment.

Dr. Wood further writes me that he obtained the same substance from the diseased portion of the bone tissue, but that he could not obtain it from the bone-marrow in any other portion of the body of the patient.

The material which I received for examination had been isolated by precipitation with alcohol, and appeared as a white, pasty mass, which on prolonged standing dissolved in water, but much more readily in very dilute ammonia (0.5 to 0.25 per cent.). This solution remained clear on subsequent neutralization; on heating a turbidity appeared between 50° C. and 55° C., which cleared on boiling and reappeared on cooling. The behavior toward dilute ammonia showed that the product was not contaminated by the common albumins which may occur in the urine. Like my own substance, this also could be precipitated from its neutral solutions by salting with magnesium sulphate to saturation, and it was noted that the precipitate was soluble in water. With rock salt partial precipitation also occurred, and in this case the precipitate was likewise soluble in water. The limits of precipitation with ammonium sulphate were 1.7 and 2.8, respectively. On digestion with pepsin-hydrochloric acid a proto-albumose was formed which could be precipitated by one-half saturation with ammonium sulphate, and which was soluble in 66 per cent. alcohol. A hetero-albumose could not be demonstrated; but, as in the first instance, I also obtained the three deutero-albumoses A, B, and C, and the two peptones A and B, though of these the deutero-albumose B and peptone B were the only products that were present in large amounts.

SUMMARY OF REPORTED CASES. It had been my intention to present a more detailed account of the various cases of Bence Jones' "albumosuria" that have been reported in the literature, but it was found that such an analysis would have occupied more space than was available. I therefore abandoned the idea, and have below merely arranged the reported cases in chronological order, together with the necessary references and the anatomical diagnosis whenever a record of an autopsy could be found.

The *first case* occurred in the practice of Drs. Watson and MacIntire, and was reported by Bence Jones,[1] in 1847, before the Royal Society of London. A clinical report of the case by MacIntire followed in 1850. The post-mortem diagnosis was "osteomalacia fragilis rubra."

The *second case* occurred in the practice of M. Doornik, of Amsterdam, in 1869, and was apparently also seen by Stokvis, who examined the urine. The clinical diagnosis in this case also was osteomalacia, but a post-mortem examination was not made. The urine was studied in detail by Kühne,[2] who published his results in 1883.

The *third case* was described from the clinical stand-point by Kahler in 1889. The clinical diagnosis in his case also had been osteomalacia, but post-mortem multiple myeloma was discovered. Kahler suggested that had a detailed microscopical examination been made in the two other cases it might have been found that they also were in reality cases of multiple myeloma which clinically simulated osteomalacia. He expressed the opinion that the presence of Bence Jones' "albumose" might possibly be of service in the diagnosis of multiple myeloma from osteomalacia. The urine in Kahler's case was examined by Huppert.[3]

The *fourth case* occurred in the service of Stokvis,[4] of Amsterdam. A preliminary report appeared in 1891. A more detailed account of the case was given by Ribbink in 1892, and in 1893 Zeehuisen published his report of the autopsy together with the chemical examination of the diseased tissues. The anatomical diagnosis was osteosarcomatosis (myeloma).

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[1] H. Bence Jones. Philos. Trans. Royal Soc., 1848, Pt. I. p. 55. W. MacIntire. Med. Chir. Trans., London, 1850, vol. xxxiii. p. 211.

[2] W. Kühne and R. H. Chittenden. Ueber die nächsten Spaltungsprodukte d. Eiweisskorper, Zeit. f. Biol., 1883, vol. xix. p. 198. W. Kühne, Ueber Hemialbumose in Harn, ibid., p. 209. W. Kühne and R. H. Chittenden, Ueber Albumosen, ibid., 1884, vol. xx. p. 40.

[3] Huppert. Ueber einen Fall von Albumosurie, Prag. med. Woch., 1889, vol. xiv. p. 35.

[4] B. I. Stokvis. Ueber Hemialbumosurie, Nederl. Tijdschrift voor Geneesk., 1891, vol. ii. p. 36, cit. in Maly's Jahresb., 1892, vol. xxi. p. 412. H. C. G. Ribbink, Een geval v. Albumosurie, Diss. Amsterdam, 1892, cit. in Maly's Jahresber., 1893, vol. xxii. p. 525. H. Zeehuisen, Nederl. Tijdschr. v. Geneesk., 1893, Pt. I. p. 829, cit. in Maly's Jahresber., 1894, vol. xxiii. p. 577.

The *fifth case* occurred in Stintzing's clinic in 1895. The clinical history, together with an account of some experiments regarding the general metabolism of the patient, is reported by Seegelken.[1] The study of the urine was made by Matthes in Neumeister's laboratory. Post-mortem examination revealed the existence of multiple myeloma.

The *sixth case* occurred in the clinic of Senator,[2] who has reported the history of the patient in detail. A general account of the patient's urine is given by Rosin, while a more detailed study appears in Süssmann's thesis of 1897 (Leipzig). Post-mortem myelogenous round-celled sarcomatosis (myeloma) of the ribs was found.

The *seventh case* is reported by Bozzolo[3] under the interesting title "Sulla malattia di Kahler." The urine was examined by Belfanti. An autopsy could not be made, but it is quite clear from the detailed clinical account which is given that this patient also was the subject of multiple myeloma.

The *eighth case* is reported by Ewald.[4] During the life of the patient the diagnosis of myeloma was made upon the basis of a histological examination of a growth of the right clavicle which was removed at an operation.

The *ninth case* occurred in Lichtheim's clinic, and is reported by Ellinger.[5] The anatomical diagnosis was multiple lymphoma (myeloma) and lymphoid infiltration of the bone-marrow of the ribs, the vertebræ, and sternum.

The *tenth case* was briefly reported by Naunyn,[6] in 1898, at a meeting of the "Unter-Elsässischer Aerzteverein," in Strassburg (January 29, 1898). He expressed the opinion that in this case, also, multiple myeloma of the bones of the thoracic skeleton existed, but an autopsy was not made. The urine was studied in detail by Magnus-Levy.

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[1] Seegelken. Ueber multiples Myelom u. Stoffwechseluntersuchungen b. demselben, Deut. Arch. f. klin. Med., 1897, vol. lviii. p. 276. M. Matthes, Ueber Eiweisskörper im Urine b. Osteomalacie, Verhandl. des XIV. Cong. f. inn. Med., 1896, p. 476. R. Neumeister, Lehrbuch. d. physiol. Chem., 1897, 2d ed., p. 806.

[2] H. Senator. Asthenische Lähmung, Albumosurie u. multiple Myelome, Berl. klin. Woch., 1899, vol. xxxvi. p. 161. H. Rosin, Ueber einen eigenartigen Eiweisskörper im Harn, u. seine diagnostische Bedeutung, Berl. klin. Woch., 1897, vol. xxxiv. p. 1044.

[3] Bozzolo. Sulla malattia di Kahler, La Clinica medica Italiana, 1893, cit. in Centralbl. f. d. med. Wiss., 1898, vol. xxxvi. p. 572. The case was first reported in 1897 before the Eighth Congress of International Medicine.

[4] K. Ewald. Ein chirurgisch interessanter Fall v. Myelom., Wien. klin. Woch., 1897, vol. x. p. 169.

[5] A. Ellinger. Das Vorkommen des Bence Jones'schen Körpers im Harn bei Tumoren d. Knochenmarkes u. seine diagnostische Bedeutung, Diss. Königsberg, 1898, and Deutsch. Arch. f. Klin. Med., 1899, vol. lxii. p. 255.

[6] Naunyn. Ein Fall v. Albumosurie, Deutsch. med. Woch., 1898; Vereins Beilage, No. 30, p. 217. Magnus-Levy, Ueber d. Bence Jones'schen Eiweisskörper, Zeitschr. f. physiol. Chem., 1900, vol. xxx. p. 200.

The *eleventh case* was briefly reported by Bradshaw[1] before the Royal Medical and Chirurgical Society, April 26, 1898. A further report appeared in 1900. No record of an autopsy is given, but Bradshaw regards the case as an unmistakable instance of bone disease and probably of myelomata.

The *twelfth case* is reported by Fitz.[2] The clinical diagnosis was myxœdema, and there was no clinical evidence of bone disease. The patient died while under treatment with thyroid extract. Unfortunately, an autopsy could not be held. The urine was examined by Dr. E. S. Wood.

The *thirteenth case* also was under the care of Dr. Fitz,[3] and is briefly referred to in his previous paper, where he speaks of the patient as being then under the charge of Dr. F. C. Shattuck. An account of the clinical history of the case and a study of the post-mortem findings is given by Wright. The urine in this case also was examined by Dr. E. S. Wood, and through the latter's courtesy I was enabled to study the isolated substance. (See above.)

The *fourteenth case* is reported by Vladimir de Holstein,[4] in 1898. In this case the diagnosis "multiple myelomatosis" was made during the life of the patient on the basis of the existing "albumosuria." In a subsequent note it is stated that the clinical diagnosis was confirmed by autopsy. A detailed examination of the urine is wanting.

The *fifteenth case* is reported by Buchstab and Schaposchnikoff.[5] The original diagnosis had been pseudoleukæmia, but after Bence Jones' "albumose" was found in the urine it was changed to myeloma. This was confirmed by autopsy.

The *sixteenth case* occurred in the clinic of Lichtheim, and was reported by Askanazy[6] before the Verein f. Wissenschaftliche Heilkunde, in Königsberg (Prussia), at the meeting of January 23, 1899. A more detailed account of the case appeared in 1900. Post-mortem examination showed the existence of lymphatic leukæmia, with the corresponding characteristic changes in the bone-marrow.

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[1] T. R. Bradshaw. A Case of Albumosuria, Brit. Med. Journ., 1898, Pt. I. p. 1136. Myelopathic Albumosuria, ibid., November 3, 1900.

[2] R. H. Fitz. The Significance of Albumosuria in Medical Practice, THE AMERICAN JOURNAL OF THE MEDICAL SCIENCES, 1898, vol. cxvi. p. 30.

[3] THE AMERICAN JOURNAL OF THE MEDICAL SCIENCES, ibid., p. 43. J. H. Wright, A Case of Multiple Myeloma, Journ. of the Boston Soc. of Med. Sci., 1900, vol. iv. p. 195, and Contributions to the Science of Medicine, W. H. Welch Memorial Vol., Johns Hopkins Press, 1900, p. 359.

[4] Vladimir de Holstein. Semaine Médicale, 1898, p. 206, and ibid., 1899, p. 83.

[5] L. Buchstab and B. Schaposchnikoff. Ein Fall von diffusem Myeloma d. Knochen in Zusammenhange mit einer typischen Albumosurie als einem charakteristischen diagnostischen Merkmale. Russ., Arch. f. Pathol., klin. Med. und Bacteriol., 1899, vol vii. p. 11, Russian.

[6] S. Askanazy. Ueber die diagnostische Bedeutung d. Bence Jones'schen Albumosurie, Deutsch. med. Woch., 1899, vol. xxv. V. B. p. 177, and Deutsch. Arch. f. klin. Med., 1900, vol. lxviii. p. 34.

The *seventeenth case* is briefly described by Sternberg,[1] in 1899. An autopsy was not made. In the urine large amounts of the Bence Jones substance were found (examination by E. Freund). An account of the reactions of the body, however, is not given.

The *eighteenth case* is the one under present consideration. A preliminary report was made by Dr. Hamburger[2] before the meeting of the Johns Hopkins Medical Society on November 5, 1900. The clinical diagnosis, as I have said, was multiple myeloma, but an autopsy, unfortunately, could not be made.

The *nineteenth case* occurred in the service of Dr. Osler, and is likewise reported by Hamburger.[3] Autopsy revealed myelomata in the skull, left scapula, both clavicles, the sternum, the right ilium, and neck of the right femur. A detailed report of the post-mortem findings is given by MacCallum.[4]

The *twentieth case* is reported by Kalischer.[5] Post-mortem examination revealed lymphoid hyperplasia of the marrow of the ribs (myeloma). (Examination by Dr. Franz Müller.)

The *twenty-first case* is briefly referred to by Rostoski.[6] An account of the clinical history or of a post-mortem examination is not given.

The *twenty-second case*, finally, is reported by Jochmann and Schumm.[7] The writers make no mention of any of the cases that have been reported since 1897, with the exception of a supplementary note on the preceding case. They claim that their case is the first true instance of osteomalacia and not of multiple myeloma, in which the Bence Jones substance was found in the urine. Autopsy showed involvement of the vertebræ, the ribs, sternum, pelvic bones, and long bones. In addition, interstitial and parenchymatous nephritis was noted, with slight amyloid degeneration.

The above twenty-two cases are the only ones recorded in the literature in which the presence in the urine of the Bence Jones substance has been definitely established. It will be noted that, barring those in which no autopsy was made, and assuming, with Kahler, that Bence Jones' case was probably myeloma and not osteomalacia, myeloma actually existed in thirteen. The relation between the two conditions in so large a percentage of cases suggested the possibility that the substance may possibly have been present in still other cases of myeloma also, but may not have been recognized as such. Unfortunately, the term myeloma is not applied to a definite clinical picture, nor even to a uniform pathological-histological condition, so that I was much embarrassed in my search of the literature in deciding what cases of apparent malignant disease of the bones belonged to this category. I was greatly aided, however, by the data given in Wieland's[1] excellent paper, although the writer's interpretation of some of our cases in which the Bence Jones subtance was found in the urine is at variance with the diagnosis originally made. The cases which Wieland accepts as cases of true myeloma, in addition to the thirteen already referred to, are those of v. Rustitzky,[2] Zahn,[3] and Klebs,[4] even more probably that of Weber,[5] possibly the case of Marchand,[6] and perhaps also those of Winkler[7] and Funkenstein.[8] Markwald's[9] case, which was formerly regarded as an instance of myeloma, is now classified as endothelioma. I have gone over the original report of the case, together with the true myeloma cases, however, notwithstanding, as it was very similar to that of Kahler,[10] in which the Bence Jones substance was actually found. My search, however, has not led to the discovery of a new, well-defined case of Bence Jones' "albumosuria." Zahn, it is true, reports that in his case albumin was always present in variable amounts, and post mortem no definite lesion of the kidneys was found. A more detailed account of the reactions, however, is lacking. v. Rustitzky, Weber, Winkler, and Markwald remark that in their cases no albumin was present in the urine, and v. Jaksch[11] further states that in one case of myeloma which he observed the Bence Jones albumin could at no time be demonstrated. Marchand makes no note of a urinary examination whatever. As a result we are forced to the conclusion that while the Bence Jones albumin may be present in association with myeloma, this is not necessarily the case. There is some evidence to show, moreover, that the substance may occur in other diseases affecting the bone-marrow as well. I have thus pointed out that in Jochmann and Schumm's case post-mortem examination revealed the existence of osteomalacia, and in Askanazy's case of lymphatic leukæmia (lymphadenoid degeneration of the bone-marrow). A detailed report of the post-mortem findings in Jochmann's case has not as yet appeared; but if future investigations should prove that their case was actually one of osteomalacia, the observation would gain in interest, as other observers have heretofore not found the substance in the disease in question, although diligent search has been made. Bence Jones himself thus writes to Kühne that he examined the urine of a fairly large number of well-marked cases (from 1845 to 1869), but that he could not find the substance again.

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[1] M. Sternberg. Vegetationsstörungen und Systemerkrankungen der Knochen, Nothnagel's Pathol. und Therap., 1899, vol. vii., pt. 2, sec. 2, p. 57.

[2] L. P. Hamburger. Loc. cit.

[3] Loc. cit.

[4] W. G. MacCallum. A Case of Multiple Myeloma, Journ. Exper. Med., 1901, vol. vi. p. 53.

[5] S. Kalischer. Ein Fall v. Ausscheidung d. Bence Jones'schen Eiweisskörper durch d. Urin bei Rippenmyelomen, Deutsch. med. Woch., 1901, vol. xxvii. p. 54.

[6] Rostoski. Albumosurie und Peptonurie, Münch. med. Woch., 1901, vol. xlviii. p. 1115. Proceedings of the Physikalisch-medicinische Gesellsch. v. Würzburg, meeting of June 13, 1901.

[7] G. Jochmann und O. Schumm. Typische Albumosurie bei Osteomalacie, Münch. Med. Woch., 1901, vol. xlviii. p. 1310.

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[1] E. Wieland. Studien über das primär multiple auftretende Lymphosarcom der Knochen. Virchow's Arch., 1901, vol. clxvi. p. 103.

[2] v. Rustitzky. Multiples Myelom. Deutsch. Zeitsch. f. Chirurg., 1873, vol. iii.

[3] F. W. Zahn. Ueber das multiple Myelom, seine Stellung im onkologischen System u. seine Beziehung zur Anæmia lymphatica, Deutsch. Arch. f. Chirur., 1885, vol. xxii.

[4] Klebs. Die allgem. Pathol., 1889, Pt. 2.

[5] Weber. General Lymphadenomatosis of Bones, One Form of "Multiple Myeloma," Journ. of Pathol. and Bacter., 1898, p. 59.

[6] Marchand. Ein Fall von seniler Osteomalacie, Berlin. klin. Woch., 1889, No. 29, p. 486.

[7] Winkler. Das Myelom. in anatom. u. klin. Beziehung, Virchow's Arch., 1900, vol. clxi. p. 252.

[8] O. Funkenstein. Ein Fall von multiplem Myelom, Inaug. Diss , Strasburg, 1900.

[9] Markwald. Ein Fall von multiplem, intervasculärem Endotheliom in d. gesammten Knochen d. Skelets, Virchow's Arch., 1895, vol. cxli. p. 128.

[10] Loc. cit.

[11] v. Jaksch. Verhandl. d. 18th Cong. f. inn. Med., 1900, p. 500.

Stokvis, also, has not a single instance to report where Bence Jones' "albumosuria" was associated with osteomalacia, and v. Jaksch[1] states that he never found "albumose" in the urine in numerous cases of the disease. The only other observations which I have been able to find in the literature in which it is claimed that "albumoses" were present in the disease in question are the cases of Langendorff-Mommsen[2] and of Raschkes.[3] In neither case, however, is there sufficient evidence to show that the urine actually contained the Bence Jones substance, although the reactions described by Raschkes suggest it rather more strongly than in the first case.

In association with other multiple new-growths affecting the bones, excepting myeloma, the substance in question has apparently not been found, unless, indeed, some of the cases which have been reported under the title "myeloma" are, as Wieland and others suggest, not cases of true myeloma. Into this question, however, I can scarcely enter at this place. Suffice it to say that an analysis of the reported cases of multiple tumors affecting the bones, which I have made with a view of possibly discovering some overlooked instance of "albumosuria," led to no result. Runeberg,[4] it is true, reports that in his case the urine contained *much* albumin, and at the autopsy it was noted that in the spongy portion of the sternum, the ribs, the vertebræ, and the sacrum there was a marked hyperplasia of granular, light yellow, nucleated cells, as also a large number of "giant cells." Runeberg states that the urine contained numerous hyaline and finely granular casts, and adds that the finding of the latter alone speaks against a true inflammatory disease of the kidneys. The existence of cirrhotic kidneys, moreover, was excluded, and at the post-mortem it could be definitely shown that a nephritis did not exist. From the account given the possibility thus suggests itself that in this case the "albumose" may have been present, but a definite conclusion can scarcely be reached in the absence of a more detailed examination.

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[1] R. v. Jaksch. Klinische Diagnostik, 1896, Wien u. Leipzig.

[2] O. Langendorff and J. Mommsen. Beiträge zur Kenntniss d. Osteomalacie, Virchow's Arch., 1877, vol. xlix. p. 649.

[3] A. Raschkes. Ein Fall von seniler Osteomalacie mit Albumosurie, Prag. med. Woch., 1894, vol. xix. p. 649.

[4] J. W. Runeberg. Ein Fall von medullärer Pseudoleukæmie, Deutsch. Arch., 1883, vol. xxxiii. p. 629.

The negative cases, in regard to which I consulted the original histories, are those reported by Lannelongue,[1] Arnold,[2] Litten,[3] Grawitz,[4] Waldstein,[5] Hammer,[6] Wieland,[7] Kudrewetzky,[8] and Nothnagel.[9]

In cases of multiple metastatic carcinoma involving the bones "albumosuria" never occurs.

The only instance of bone disease other than what has been described above, in which a substance has been encountered in the urine of man, which may be interpreted as being identical with the body under consideration, is a case of tubercular osteo-arthritis of the shoulder that has been briefly reported by Vidal[10] before the Société de Biologie of Paris (October 29, 1898). The patient died of acute pleuro-peritoneal tuberculosis, but an autopsy was apparently not held. The reactions which were obtained from the patient's urine, and from which Vidal himself concludes that the substance must have belonged to the "primary proteoses," are the following: After the addition of a few drops of acetic acid no precipitate results on boiling. A well-marked biuret reaction is obtained. Cold nitric acid produces a milky turbidity, which disappears on boiling and reappears on cooling.[11]

It is stated by Laache[12] and Lussana[13] that in pernicious anæmia "peptonuria," in the older meaning of the term, is of common occurrence; but I have not been able to find any data in the literature to warrant the conclusion that the "peptone" in such cases is identical with the Bence Jones albumin. The statement, however, is of interest, as it is a well-known fact that myeloma may follow a clinical course which is quite similar to the common form of pernicious anæmia. A more careful search in this particular direction than I have been able to make may possibly unearth still other cases of Bence Jones' albuminuria.

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[1] Lannelongue. Gaz. des Hôpit., Nos. 41 and 42.

[2] Arnold. Drei Fälle v. primärem Sarcom d. Schädels, Virchow's Arch., 1873, vol. lvii. p. 297.

[3] Litten. Ueber einen in medulläre Leukæmie übergehenden Fall v. primärer Anæmie. Berlin. klin. Woch., 1877, Nos. 19 and 20.

[4] P. Grawitz. Maligne Osteomyelitis u. sarcomatöse Erkrankungen d. Knochensystems als Befunde b. Fällen v. perniciöser Anæmie, Virchow's Arch., 1879, vol. lxxvii. p. 353.

[5] L. Waldstein. Ein Fall von progressiver Anæmie u. darauffolgender Leucocythæmie mit Knochenmarkerkrankungen u. einem sogenannten Chlorom (Chlorolymphom), Virchow's Arch., 1883, vol. xci. p. 12.

[6] Hammer. Primäre sarcomatöse Ostitis mit chronischem Rückfallsfieber, Virchow's Arch., 1894, vol. cxxxvii. p. 280.

[7] Wieland. Loc. cit.

[8] B. Kudrewetzky. Zur Lehre v. d. durch Wirbelsäulen Tumoren bedingten Compressions, Erkrankung d. Rückenmarks, Zeitsch. f. Heilkunde, 1892, vol. xiii.

[9] H. Nothnagel. Ueber eine eigenthümliche perniciösen kockenerkrankung. (Lymphadenia ossium), Virchow's Festschrift, 1891, vol. ii. p. 155.

[10] E. Vidal. Note sur un cas d'albumosurie, Compt. rend. de la Soc. de biol., 1898, vol. l., p. 991.

[11] I find that another case is briefly reported in which the Bence Jones substance was apparently present in the urine. No details, however are given. Matthes, Verhandl. d. Congr. f. inner Med., 1900, p. 501.

[12] Laache. Die Anæmie. Christiania, 1883.

[13] Lussana. Cit. by Eichhorst, Perniciöse Anaemie, Eulenburg's Real-Encycl., 3d ed.

From the reported cases of Bence Jones' albuminuria the conclusion may be drawn: 1. That the presence of the substance in the urine of man usually indicates the existence of multiple new-growths affecting the bones, and generally of myeloma. 2. That myeloma may apparently occur in the absence of the peculiar albuminous substance. 3. That the existence of the urinary condition in association with other diseases without involvement of the bones is not as yet proved by the report of any case in which a careful post-mortem examination has been made. 4. That malignant disease of the bones, in general, may exist in the absence of the Bence Jones albumin from the urine.

Outside of the urine, and possibly the blood and the tumor masses of the cases described in the foregoing pages, the Bence Jones albumin has apparently also been encountered in the urine of dogs during the continued administration of pyrodin (monoacethyl-phenylhydrazin). Zülzer[1] states that the "albumosurie" is practically a constant result of pyrodin poisoning in dogs, but that it only persists in its pure form for a very short time, and is soon obscured by the common form of albuminuria, which develops as the result of the inevitable nephritis. He cites one instance, however, in which the albumosuria persisted for four days, uncomplicated by albuminuria. With nitric acid a heavy precipitate occurred at ordinary temperatures, which dissolved entirely on heating. Similar results were obtained with sulpho-salicylic acid, picric acid, Almen's reagent, and acetic acid and potassium ferrocyanide. On heating the urine, after acidifying distinctly with acetic acid, a marked turbidity occurred between 50° C. and 60° C., which disappeared almost entirely on heating to 100° C. The isolated substance (precipitation with alcohol) gave the same reactions as the native urine. After drying it was soluble only in boiling water after the addition of a little soda. Millon's reaction, the biuret reaction, and the sulphur test were positive. On half saturation with salt a marked turbidity occurred (!).

A detailed account of the experiments is still lacking, but I conclude from Zülzer's paper that the "albumosuria" was associated with changes in the bones.

In Tallquist's[2] monograph on pyrodin-anæmia no mention is made of the occurrence of "albumosuria," but it is possible, as Zülzer suggests, that it has been overlooked, owing to the simultaneous presence of common albumin.

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[1] G. Zülzer. Ueber experimentelle Bence Jones'sche Albumosurie, Berlin. klin. Woch., 1900, vol. xxxvii. p. 894.

[2] T. W. Tallquist. Ueber experimentelle Blutgift-Anaemien. Berlin, 1900. A. Hirschwald.

Whether or not the Bence Jones substance may also occur in normal bone-marrow, as is suggested by the experiments of Fleischer,[1] remains to be decided by future researches according to more modern methods. If so, it is apparently only present in traces. Larger amounts of a similar but apparently not identical substance were found by Virchow[2] in the bone-marrow of a patient dead with osteomalacia; and it has been stated by some that this was in reality the Bence Jones albumin. Virchow, however, has made no statement to this effect; and Kühne in speaking of this observation states that it merely shows "that common albumin after solution in acetic acid by boiling can give rise to a precipitate, on treating with nitric acid, which dissolves on heating and reappears on cooling." Fleischer's substance differs from that of Virchow in the fact that the precipitate caused by nitric acid dissolved upon the further addition of concentrated acetic acid.

COMPARISON OF RESULTS. *Amount.* On comparing my results with those obtained by others, and these in turn with each other, it is clear, I think, that all observers have been dealing with one and the same substance. They all mention that on treating the urine with nitric acid a precipitate results, which disappears on boiling and reappears on cooling. This reaction, it is true, is not characteristic of the Bence Jones substance, as it is also obtained with the deutero-albumoses which may occur in the urine under various other pathological conditions. But it should be borne in mind that whereas deutero-albumoses are usually only present in such small amounts that special methods are necessary for their demonstration, the Bence Jones substance generally occurs in quantities which are so large as to arrest attention at once. In the following table I have collected the results which have been obtained by the various observers:

Fitz	0.12 —	0.93	pro mille.
Ellinger	0.25 —	0.5	" "
Askanazy	0.5 —	1.25	" "
Fitz	0.1 —	2.25	" "
Huppert	3.0		" "
Fitz-Wright	3.3 —	3.4	" "
Kalischer	5.0 —	5.0	" "
Süssmann	6.0		" "
Hamburger, I.	6.0		" "
Matthes	4.0 —	6.0	" "
Simon	0.12 —	5.12	" "
Bozzolo	10.0		" "
Bradshaw	10.0		" "
Ribbink	20.0		" "
Magnus-Levy	18.0 —	24.0	" "
Bence Jones	67.0		" "
Noël Paton	70.0		" "

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[1] R. Fleischer. Ueber das Vorkommen d. sogenannten Bence Jones'schen Eiweisskörper's im normalen Knochenmark, Virchow's Arch., 1880, vol. lxxx. p. 482.

[2] R. Virchow. Ueber parenchymatöse Entzündungen, Virchow's Arch., vol. iv. p. 308.

From this table it will be seen that in some cases the elimination of the substance far exceeds the largest amounts of common albumin that may be encountered in the urine.

Behavior on Heating. All observers agree that the albuminous substance in question begins to be precipitated from the urine at a temperature which is well below that at which serum albumin is coagulated, viz., between 50° C. and 58° C. Individual variations are undoubtedly referable to a varying degree of acidity and the amount of salt present, and may indeed occur in one and the same case. Kühne's is the only case in which the initial temperature of precipitation was found below 50° C., viz., at 43° C. Naunyn states that his substance was precipitated between 49° C. and 53° C. As regards the behavior of the urine on further heating, the statements of the various observers differ more radically. Kühne and Matthes thus find that in their cases the urine cleared entirely on boiling, and it appears that this also occurred in Ellinger's case, as the faint turbidity which remained at 100° C. was here no doubt referable to the contaminating nucleo-albumin. In Ribbink's case and those of Huppert, Rosin, Magnus-Levy, Rostosky, and myself, on the other hand, the urine did not clear on boiling. This apparent difference might suggest that the substance which the various investigators had under observation was not the same in all cases. Such a conclusion, however, is not admissible, in view of the fact that all possible variations in the solubility of the precipitate at 100° C. may be observed not only in the different cases, but even in one and the same instance. Naunyn thus reports that the precipitate which appeared between 49° C. and 53° C. cleared almost entirely on subsequent heating to boiling. Magnus-Levy, who studied the urine from the same patient later on, writes that at first a large portion of the precipitate dissolved on boiling, but that a considerable fraction remained undissolved; and still later in the course of the disease it was found that even with a neutral reaction the substance could be quantitatively precipitated by heat and remained insoluble on boiling. In my case, also, it was found that at first the urine became "clear" on boiling after previous precipitation; while later, when I had occasion to study the urine, a clearing at 100° C. could scarcely be noted with the eye. I have shown, moreover, that in my case, as in Magnus-Levy's, it was possible to *completely* precipitate the substance by heat if the elevation of temperature was carried out slowly, providing that a sufficient amount of salt was present and the urine presented an acid reaction. In such an event no solution whatever occurred on boiling. But, aside from these observations, the identity of the various substances is proved by the fact that, as Magnus-Levy first pointed out, it is possible to render the body soluble at 100° C., or insoluble, at will, by simple measures which in themselves can hardly be supposed to change the chemical nature of the substance. A solution of the isolated substance, moreover, which has been rendered acid by acetic acid, and which contains a certain amount of salt, shows the "typical" behavior of the Bence Jones substance on heating as it was described by Kühne. Its insolubility at 100° C. in the urine must hence depend upon the associated presence of some other substance or substances in the urine, among which the salts, no doubt, are of prime but not of exclusive importance. What other factors are here of moment, however, is as yet unknown. Remembering the influence of the degree of acidity upon the phenomenon, it might be supposed that the relative amount of acid phosphates would enter into consideration, but this does not seem to be the case. Using two different mixtures of monopotassium phosphate and disodium phosphate in solution, of which the one was strongly alkaline, while the other was markedly acid, and adding varying amounts of these solutions to a given amount of a solution of the Bence Jones substance, Magnus-Levy was unable to obtain any effect whatever upon the coagulability of the body. He showed, however, that the addition of ammonium chloride in a definite proportion (½ per cent.) renders the substance more readily soluble at 100° C. after it has been previously coagulated, and that the addition of a certain amount of urea to the solution or to the urine may prevent heat coagulation altogether. These experiments I have repeated, and I have been able to confirm Magnus-Levy's results. I do not think, however, that the differing behavior of the urine, as noted by different observers, can be explained upon the basis of these observations alone. Other factors are here, no doubt, active, of the nature of which we are as yet in ignorance; and to the activity of such factors I have no doubt some other and minor variations in the behavior of the body that have been noted by different observers may be due. For such reasons, also, differing reactions may be obtained in one and the same case at different times. So much is certain, however, that it is no longer admissible to speak of the solubility of the coagulated substance at 100° C. as a characteristic and typical reaction, *so far as the urine is concerned. It is characteristic, however, of a solution of the isolated substance in the presence of a certain amount of acid and of salt. As the most characteristic general reaction, I should regard the coagulability of the substance at a temperature of from 50° C. to 58° C.*[1]

In the above pages I have repeatedly made use of the term *coagulation* in reference to the precipitation of the Bence Jones substance by heat or on treating with alcohol. Strictly speaking, this term is not applicable, however, as it implies a material alteration of the chemical properties of the substance, which in reality has not been effected. It is true that such precipitates are practically insoluble in cold distilled water, but they dissolve with comparative ease in boiling water in the presence of a small amount of sodium carbonate, and, as Magnus-Levy has first pointed out, the alcohol precipitate is readily soluble in very dilute solutions of ammonia. On subsequent neutralization a solution is then obtained which behaves, on heating, as the original solution, if ammonium chloride is present to the extent of ½ per cent. The latter observation is especially important, as the common albumins which may occur in the urine are insoluble in dilute ammonia after having been precipitated by alcohol. The coagulate, moreover, is readily soluble in dilute acids, and from such solutions the substance is again thrown down upon the application of heat, providing that a certain amount of salt is present.

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[1] Whether or not serum albumin was actually present in the urine in association with the Bence Jones substance as often as stated by individual observers I am not prepared to decide. I should suggest, however, that in the future a special examination in this direction be made. To conclude that the common albumins are present because the urine does not clear entirely on boiling is unwarrantable.

Dialysis. As regards the behavior of the substance on dialysis, all observers who have examined in this direction agree that it does not pass through parchment, with the exception of Ribbink, who states that a portion of his substance escaped in this manner. It is quite possible, however, that Ribbink's parchment was not altogether dense. Important in this connection is the fact that the substance is not precipitated from its solutions by dialysis, no matter how prolonged this may be.

Behavior on Salting with Sodium Chloride. Apparent differences are recorded in the behavior of the substance on salting with sodium chloride. Huppert reports that in his case the substance was completely precipitated from the urine by salting to saturation between 35° C. and 40° C. Ribbink, on the other hand, reports that even after standing for several days he was unable to obtain a precipitate by salting with rock salt at any temperature between 15° C. and 40° C. Matthes states that in his case only a fraction of the substance was precipitated by salting the urine to saturation, and then only on standing for several days. Süssmann and Magnus-Levy found that in their cases the substance could be partially precipitated from the acid urine in this manner, and Ellinger writes that on the addition of a saturated solution of sodium chloride he obtained a precipitate even at ordinary temperatures. Kühne, under the latter conditions, merely noted a turbidity. In my case no turbidity whatever occurred on treating the acid urine with a saturated solution of common salt, even when added in the proportion of 3 to 1. With an alkaline urine, however, that had undergone ammoniacal decomposition I found that a precipitate formed upon the addition of 0.4 c.c. of a saturated solution of salt to 2 c.c. of the urine. With rock salt partial precipitation occurred at ordinary temperatures, while complete separation occurred on salting at from 35° C. to 40° C.

With a neutral solution of the isolated substance I very curiously found that partial precipitation occurred on salting with sodium chloride to saturation, whereas Magnus-Levy and Süssmann state that a neutral and purified solution of the body cannot be precipitated by salting with sodium chloride at ordinary temperatures. Magnus-Levy, however, has manifestly overlooked the fact that Kühne also found that his substance could be precipitated with sodium chloride from its neutral solutions. In his second communication he states distinctly that a solution of the isolated substance gave a copious precipitate upon the addition of rock salt prisms in acid, as well as in neutral and feebly alkaline solutions. The precipitate, moreover, was soluble in water.[1]

These data in themselves suffice to show that the question, whether or not the Bence Jones substance can be precipitated on salting with sodium chloride, depends not only upon the reaction of the solution, but upon other factors as well, which are as yet but little understood.

The observation of Huppert, who succeeded in completely precipitating his substance from the urine by salting to saturation at a temperature between 35° C. and 40° C., may be explained by the assumption that this precipitation is not a true salt precipitation at all, but an actual coagulation, which, owing to the presence of so large an amount of salt occurs at a much lower temperature. I have accordingly also found that the precipitate, which I obtained on salting with rock salt to saturation at a temperature of about 37° C., was insoluble in water.

Limits of Precipitation with Ammonium Sulphate. The differences in the limits of precipitation with ammonium sulphate which exist between Magnus-Levy's case, Ellinger's, Fitz's No. 2, and my own are possibly owing to differences in the amount of salts which were originally present in the urine, to varying strengths of the albuminous solutions, and like factors. On this basis, at any rate, it would scarcely be warrantable to assume that each of us was dealing with a different substance.

Ellinger (solution of isolated substance, previously coagulated and dissolved in a dilute solution of sodium carbonate)	2.0	and	4.0
Magnus-Levy (similar preparation, but subsequently neutralized) .	0.6	"	2.2
Magnus-Levy (similar preparation, but dissolved in ammonia) . .	2.5	"	6.0
Magnus-Levy (neutralized urine).	4.0	"	6.0
Simon (neutralized urine)	0.7	"	4.2
Simon (solution of isolated substance, precipitated with ammonium sulphate and dialyzed for eight weeks, neutral reaction) . . .	2.3	"	3.8
Fitz-Wright [precipitation with alcohol, solution in very dilute ammonia (Simon)]	1.7	"	3.6

Formation of "Albumosate." As regards the behavior of a solution of the substance on neutralization, after the previous addition of an acid or an alkali, the reports of all observers who have examined in this direction, with the exception of Ribbink, are the same. Ribbink states that he could not obtain a precipitate on neutralization, but it is quite likely, as Magnus-Levy suggests, that he had previously not added enough acid or alkali. Kühne, who regarded the Bence Jones substance as an albumose, speaks of the apparent change which results on treating with an alkali, as due to the formation of an albumosate analogous to the albuminate in the case of the common albumins.

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[1] Kühne and Chittenden. Loc. cit., vol. xx.

Behavior on Salting with Magnesium Sulphate. In its behavior toward magnesium sulphate a marked difference apparently exists between my substance and that of Magnus Levy. For, whereas he reports that on saturating the urine with the salt in question, at the temperature of the room, no precipitation occurred, I noted in my case that after acidifying with acetic acid the substance is promptly thrown down. My notes on this point read as follows: Upon the addition of two volumes and a half of a saturated solution of magnesium sulphate to the urine no precipitation occurred, even after acidifying with acetic acid, but on saturation of the acid solution with the salt in substance, complete precipitation occurred. Saturation without the addition of acetic acid gave no result in the native urine. But on treating the neutral solution of the isolated substance (precipitation with ammonium sulphate and subsequent dialysis for eight weeks) with two or three times its volume of a saturated solution of magnesium sulphate, and then saturating with the salt in substance, the Bence Jones body was completely precipitated on standing for twenty-four hours, at the temperature of the room. The filtrate remained clear on heating after the addition of acetic acid; no further precipitate occurred on treating with twice the volume of a saturated solution of ammonium sulphate, and the biuret reaction was negative. The precipitate itself was soluble in water and could hence not be a coagulate, but must be regarded as a true salt-precipitate. Other observers have made no notes of the behavior of the substance in this respect; but it is interesting to note that the substance which Dr. Wood sent me (from Fitz-Wright case) could also be precipitated with magnesium sulphate from its neutral aqueous solution.

Behavior on Digestion. On digestion with pepsin-hydrochloric acid all observers who have made investigations in this direction report that the substance dissolves very readily, and that the presence of "peptones" can very soon be demonstrated. Matthes noted that on prolonged digestion a jelly-like material separated out, which, after drying, was soluble in absolute alcohol. This, however, as he later admitted himself, and as Neumeister indicates, was owing to the admixture of a nucleo-albumin. Ellinger records that on digestion with pepsin for forty-eight hours a small amount of residue was obtained, which contained phosphorus. In his case, however, a nucleo-albumin was also present and had not been previously removed. Speaking of the products of digestion with pepsin-hydrochloric acid, Kühne states that after one or two hours only "peptone" was found in solution, and no precipitate was obtained either with nitric acid or with acetic acid and sodium chloride.

A more detailed examination of the resulting products of digestion according to more modern methods has been made by Magnus-Levy. As I have previously indicated, he claims to have obtained a proto-albumose, three deutero-albumoses, and two peptones, while the formation of a hetero-albumose was not observed. In view of the importance of these results in their bearing on the question of the chemical nature of the Bence Jones substance, it may not be out of place to consider in some detail the basis on which Magnus-Levy has established his claim that on peptic digestion the Bence Jones substance yields a proto-albumose. Magnus-Levy states that he employed Hofmeister's method of analysis, as described by Pick,[1] Umber,[2] and Alexander.[3] According to this method the digestive mixture was neutralized (with ammonia or ammonium carbonate), brought to the boiling-point, and then concentrated on the water-bath to about one-fifth of the original volume. On neutralization Magnus-Levy states that he never obtained a precipitate, but that during the process of concentration flocculi separated out, which may have been due to acid albumin, but were present in too small a quantity for analytical purposes. On treating the filtered solution with an equal volume of a saturated solution of ammonium sulphate a precipitate then resulted, which Magnus-Levy regards as due to proto-albumose exclusively, as a hetero-albumose could not be obtained from this fraction, either on dialysis or on treating the aqueous solution of the precipitate with half and an equal volume of alcohol and boiling for several hours. He then further *identified* this fraction as proto-albumose by the following reactions, which he compares with those of the Bence Jones substance:

	Sulphur test.	Millon's reaction.	Biuret reaction.	Molisch's test.	Adamkiewicz test.	Salting with rock salt.	Rock salt and acetic acid.	Dilute copper sulphate solution.
1. Bence Jones substance	Markedly black color.	Intense.	Violet-red.	Faint but distinct.	Very pronounced.	No precipitation.	Complete precipitation.	Marked precipitation.
2. Proto-albumose	Markedly black color.	Markedly red.	Purely red.	Much more marked than under 1.	Faint.	Precipitation.	Precipitation more marked than with rock salt.	Marked precipitation.

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[1] Loc. cit.

[2] Loc. cit.

[3] F. Alexander. Zur Kenntniss d. Caseins u. seiner peptischen Spaltungsprodukte, Zeit. f. phys. Chem., 1898, vol. xxv. p. 411.

The limits of the precipitation were 3.2 and 4.8 in one test and 2.8 and 4.8 in a second.

The question which of course suggests itself on reading Magnus-Levy's paper is the following: Is it not possible that the proto-albumose, so called, was in reality a portion of the Bence Jones substance which had escaped digestion? I must confess that Magnus-Levy's account of his work is not sufficiently detailed as to render such a supposition unwarrantable. He states that after two to three hours, at the latest, the original substance had entirely disappeared, and that it was then already possible to demonstrate the presence of all the various fractions which he claims to have obtained. He does not state, however, in what manner he showed that the substance was no longer present, supposing that the substance had not been digested entirely. However, in that case it would certainly have remained in solution on neutralization and boiling, as the amount of acid previously added is scarcely sufficient to insure the precipitation of the substance on subsequent neutralization; and from the neutral solution the body would not have been precipitated on boiling. Even on acidifying again I have noted that the solution remains clear or becomes at best but slightly opalescent.

On the other hand, it might be argued that the behavior of the isolated proto-albumose, as compared with the Bence Jones substance, on salting with ammonium sulphate, as also in its general reactions, as tabulated above, would warrant the identification of the "proto-albumose" as a distinct substance. This I am willing to admit to a certain extent; but I have considered it necessary, nevertheless, to attempt to prove even more conclusively that the Bence Jones substance actually yields a proto-albumose on peptic digestion. To this end the experiments already detailed were undertaken, and the results show conclusively that a proto-albumose is actually formed, and my own work thus entirely confirms that of Magnus-Levy. A hetero-albumose is not formed. This was already suggested by Magnus-Levy's observation that on hydrolytic decomposition with hydrochloric acid and sulphuric acid the Bence Jones substance does not yield glycocoll, but furnishes a relatively large amount of tyrosin, with but little leucin.

As regards the remaining products of peptic digestion which may be obtained from the Bence Jones substance, I also have found bodies corresponding to Pick's deutero-albumoses A, B, and C. A and C, however, and notably the latter, were manifestly only produced in very small amounts, while B was present in fairly large quantity. Peptone A also was certainly only present in traces, if at all, while I could demonstrate a considerable amount of peptone B. Magnus-Levy states that his deutero-albumose B showed a minimal Molisch reaction; I obtained none whatever. However, Magnus-Levy himself has placed a sign of interrogation behind his (minimal).

CHEMICAL NATURE OF THE BENCE JONES SUBSTANCE. Regarding the chemical nature of the Bence Jones substance, it is clear from the foregoing considerations that neither Magnus-Levy nor I regard the body as an albumose, and I note that Rostoski also has come to this conclusion, though he does not cite any experiments as basis for his belief.

Kühne regarded the insoluble (in neutral saline solution), coagulated substance as dysalbumose, and supposed this to have resulted from hetero-albumose, in which form the body must have been present in the urine in solution. In addition, he suggests that deutero-albumose and proto-albumose may also have been present in traces, so that, according to Kühne, the Bence Jones substance represented a mixture of several albumoses. Huppert likewise regards his body as a hetero-albumose, especially as the substance is rendered insoluble by salting to saturation with sodium chloride at 35° C. to 40° C. The remaining observers, with the exception of Magnus-Levy, Rostoski, and myself, also regarded the substance as an albumose, while admitting, however, that it does not coincide in its reactions with any one of the known digestive albumoses. The essential points of difference are given by Matthes as follows:

1. If much salt is present the coagulated substance is not *entirely* soluble on boiling, while a precipitated albumose is soluble at 100° C. no matter how much salt may be present.

2. An aqueous solution of the substance does not pass through animal membrane, even in traces, while both proto-albumose and deutero-albumose are dialyzable to some extent.

3. The substance in question after coagulation is still soluble on washing with distilled water, as also on dialysis, especially if this is continued until practically all salt has been removed. Hetero-albumose, on the other hand, is entirely insoluble in distilled water, and is precipitated on dialysis.

4. The substance differs from hetero-albumose in the fact that it is apparently coagulated by heat, in any concentration, in the presence of a small amount of salt and acid, while hetero-albumose only coagulates in very concentrated solutions.

In addition to these factors the following also can be urged against the albumose nature of the substance, and especially against the assumption that the body may be a hetero-albumose:

1. The fact that the solubility of the coagulated substance at 100° C. may be changed at will, and that under certain conditions the coagulate is altogether insoluble at 100° C., even though the reaction of the urine and its chemical composition have not been altered by artificial means.

2. The fact that after precipitation with alcohol or with salt and acid

at ordinary temperatures the substance rapidly becomes insoluble in distilled water and neutral saline solutions. In this respect hetero-albumose resembles the Bence Jones substance, but it is to be noted that this change cannot be effected by heat.

3. The apparent formation of syntonin and alkaline albuminate on treating with acids and alkalies. In this respect the substance resembles the true albumins, but the precipitates which result on neutralization are not altogether insoluble, as in the case of the common coagulable albumins.

4. The Bence Jones substance contains a carbohydrate group, which is lacking in the hetero-albumose—of fibrin, at least.

5. Hetero-albumose (of fibrin) contains 17.98 per cent. of nitrogen, of which 39 per cent. exists as diamino-nitrogen; whereas the Bence Jones substance contains 15.57 per cent. of nitrogen, of which 25 per cent. are referable to diamino-nitrogen.

6. On hydrolytic decomposition hetero-albumose yields glycocoll and but little tyrosin, while from the Bence Jones substance no glycocoll can be obtained, but relatively much tyrosin.

7. On peptic digestion the Bence Jones substance yields a proto-albumose, showing that its molecule must be more complex than that of the primary albumoses.

We are thus forced to the conclusion that the substance cannot be an albumose; but in attempting to classify it among the remaining groups of proteins we meet with further difficulties. The fact that many observers have found phosphorus in the mineral ash would at once suggest that the substance might be a nucleo-albumin, but opposed to this assumption is the statement of Magnus-Levy that by previously removing the phosphates from the urine with ammoniacal magnesia mixture he was able to obtain specimens which were entirely free from phosphorus. None of the previous observers, moreover, has succeeded in demonstrating the separation of a nuclein, on peptic digestion, if care had been taken to eliminate the presence of a contaminating nucleo-albumin. Matthes, who originally regarded his substance as a nucleo-albumose, later abandoned this idea, and concluded himself that the jelly-like material which separated out on prolonged peptic digestion was referable to the admixture of a nucleo-albumin with which the Bence Jones substance had nothing to do. In Dr. Wood's case, moreover, I also succeeded in finally obtaining a product which was free from phosphorus.

In the solubility of the alcohol precipitate in dilute ammonia the substance resembles the histons, but it differs from these in the fact that the body cannot be precipitated by ammonia from its acid solutions.

The solubility of the alcohol precipitate in dilute ammonia further distinguishes the substance from the true coagulable albumins which have thus far been studied. Those, moreover, which ordinarily appear in the urine are totally insoluble at 100° C. unless the chemical composition of the urine is artificially altered, as by the addition of excessive amounts of urea, etc. Magnus-Levy has pointed out that, like casein, the Bence Jones substance lacks the hetero group, but it differs from it in the absence of phosphorus and the presence of a carbohydrate group. As his substance was not precipitated by salting with magnesium sulphate, or on passing a current of carbon dioxide through its solution, and as it proved soluble in distilled water, he concludes that the body does not belong to the class of globulins, even though in his case the limits of precipitation of the deutero-albumose A were the same as those of the corresponding deutero-albumose obtained from serum globulin. This relation and the limits of precipitation of the various other digestive products of the Bence Jones substance as compared with those of the more common albumins is shown in the accompanying table:

	Fibrin.	Egg albumin.	Serum albumin.	Serum globulin.	Bence Jones albumin.
I. Primary albumoses . . .	2.6—4.4	3.6—4.6	4.2—4.6	3.6—4.6	2.8(3.2)—4.8
II. Deutero-albumoses:					
Deutero-albumose A . .	5.4—6.2	5.6—6.0	5.4—6.2	5.6—7.2	5.6—7.2
Deutero-albumose B . .	7.2—9.5	7.0—7.8	7.2—8.0	7.8—8.6	7.8—9.2

In contradistinction to Magnus-Levy's observation, I found that my substance could be precipitated by salting with magnesium sulphate to saturation, while at the same time there was no evidence whatever that the urine contained any other albuminous body in solution but the Bence Jones substance. Other observers, as I have already stated, failed to make a note of the behavior of the body in this respect, but with the material which Dr. Wood sent me from the Fitz-Wright case No. 2 I obtained the same result.

Why my results should differ so radically in this respect from those of Magnus-Levy I am unable to say, unless, indeed, his solutions were not altogether saturated. To insure this point I followed the suggestion of Marcus,[1] and treated the solution with from two to three times its volume of a saturated solution of magnesium sulphate, and then added the salt to saturation (Marcus, p. 567). The observation, however, is important, as it suggests that the Bence Jones substance may at least be closely related to the globulins, if, indeed, it is not a globulin itself. The fact that it is soluble in water cannot be adduced as an argument to the contrary, since it has been conclusively established that even in normal blood a serum-globulin exists which is water-soluble. According to Marcus, indeed, that portion which is insoluble in water represents but 9 to 23 per cent. of the entire quantity that may be precipitated by magnesium sulphate. This water-soluble portion, as would be expected, is not precipitated by dialysis. The substance gives the xanthoproteic reaction, the sulphur and biuret reactions, that of Adamkiewicz, as also that of Molisch. An estimation of the nitrogen gave 15.9 per cent.—*i. e.*, a figure which approximates that which has been found for the Bence Jones substance, viz., 15.56 to 15.59. It is noteworthy, moreover, that in the case of a pure solution of the substance not even a turbidity is produced by means of a current of carbon dioxide, so that this point also cannot be brought forward as an argument that the Bence Jones substance should not be viewed as a globulin. On the contrary, I believe I have shown that cogent reasons exist for the assumption that the body is indeed closely related to the water-soluble globulin, as Marcus describes it. My material, however, was unfortunately not sufficient to institute a more detailed comparison between the two substances; and for other reasons also it seemed advisable to publish my results so far as they go.

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[1] E. Marcus. Ueber in Wasser lösliches Serumglobulin, Zeitsch. f. physiol. Chem., 1899, vol. xxviii. p. 559.

Should further research bear out the correctness of my view, the previously supposed identity of Noël Paton's[1] crystalline globulin with the Bence Jones substance would appear to be rendered quite probable. Huppert,[2] indeed, had previously attempted to prove their identity and at one time regarded Noël Paton's substance as an albumose; later, however, he abandoned this view,[3] and at present I think there can be no doubt that this substance also was in reality a globulin. In many respects the description of the body reminds one strongly of the Bence Jones substance. As in Bence Jones' case, it was eliminated in enormous quantities (15.0 to 70.0 pro mille, corresponding on one occasion to an elimination of 70 grammes in twenty-four hours), and, barring the Bence Jones substance, it is, indeed, the only albuminous substance which has ever been encountered in the urine in such large amounts. In this case, also, no tube-casts were found in the urine, and post-mortem examination revealed the absence of a nephritis. A further point of resemblance is the fact that Paton's globulin was obtained in crystalline form, and was deposited as such spontaneously from the urine. This occurred at times on standing for only one or two days;

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[1] Byrom-Bramwell and D. Noël Paton. On a Crystalline Globulin Occurring in Human Urine, Reports from the Laboratory of the Royal College of Physicians, Edinburgh, 1892, vol. iv. p. 47.

[2] Huppert. Ueber einen Fall von Albumosurie, Zeitsch. f. physiol. Chem., 1896-1897, vol. xxii. p. 500.

[3] Huppert. Ueber den Noël Paton'schen Eiweisskörper, Centralbl. f. med. Wissen., 1898, p. 481; cit. in Maly's Jahresber., 1899, vol. xxviii. p. 302.

at other times only after weeks or months. A solution of the crystals in neutral saline solution coagulated between 56° C. and 59° C. Very suggestive also is the following observation, which is recorded in Huppert's second communication: "The coagulum dissolves in warm water after the addition of a few drops of a sodium carbonate solution, and on neutralization with hydrochloric acid a flocculent precipitate is formed, which apparently did not dissolve upon the addition of 5 per cent. of rock salt; but on heating the filtrate a turbidity occurred at 52° C." On boiling it is stated that the urine coagulated almost in bulk, and it would thus appear that Paton's substance differed from that of Bence Jones'; but, as I have shown, the behavior on boiling cannot be regarded as a criterion in differentiating between the latter and the common coagulable albumins. In other respects, it is true, points of difference apparently exist between the two bodies; but, on the whole, these are unimportant, and I believe that we may very well classify the two together. Although future researches may show that they are not identical, there is evidence to prove even now that they are at least closely related.

The case occurred in Byrom-Bramwell's practice, while the urinary examination was conducted by Noël Paton. Evidence of bone disease did not exist during life, but it is unfortunate that post-mortem a more detailed examination could not have been made.

THE ORIGIN OF THE BENCE JONES SUBSTANCE. Of the seat of origin of the Bence Jones substance very little is known that is definite. Ellinger states that he found a substance in the blood of his patient which gave the common albumose reactions and coagulated at about the temperature of the isolated body (he indicates 38° C. to 40° C.!). Ribbink and Askanazy, on the other hand, who are the only observers who made investigations in this special direction, report negative results. Ellinger does not state whether the blood in his case was withdrawn during the life of the patient, but even if this were done the result can scarcely be surprising unless, indeed, the substance was formed in the kidneys—a supposition for which there is no tangible basis.

Askanazy states that he did not find the substance in the tumor masses, but found it in a fresh extract of the bone-marrow, which, however, was the seat of extensive lymphadenoid degeneration. Ribbink, on the other hand, records that in his case it was present neither in the bone-marrow nor in the bone-substance. Ellinger reports similar findings in the case of the tumor masses, as of the blood, and Dr. Wood writes me that he obtained the same substance, which was present in the urine, from the diseased portion of the bone tissue, and that he did not find it in the bone-marrow in any other portion of the body of

Wright's patient. Other observations on this point do not exist, but it seems to me that the findings of Ellinger and Wood more especially are very suggestive.

Most observers have been of the opinion that the Bence Jones substance originates in the diseased bone-marrow, and I must confess that Magnus-Levy's arguments to the contrary are but little convincing. He thinks that the amount of the albumin that may appear in the urine is too large to have originated in the small tumor masses. He argues as follows: "The entire amount of bone-marrow of the ribs does not exceed 200 grammes (calculated according to Dursy and Friederich in Vierordt's tables), with about one-fifth—*i. e.*, 40 grammes—of albumin. Only a portion of the marrow is replaced by myelomata. There are in addition, to be sure, the tumor masses, which may occur in other portions of the skeleton, but the combined weight of these is only very rarely notable (more than 500 grammes, corresponding to 100 grammes of albumin)—at least not in the earlier stages of the disease; and at this time the elimination of the substance, in my case, at least, reached its greatest height. It seems altogether excluded that in (relatively) such small tumor masses from 36 to 70 grammes of an abnormal albumin should daily be formed, viz., an amount which probably exceeds the weight of the dried masses themselves." Such an assumption seems to me unwarrantable, for we may readily imagine that the Bence Jones substance could result from the common albumins of the blood—such as serum globulin, for example—through enzymatic activity referable in myeloma, at least, to the plasma cells of the bone-marrow.

It would be exceedingly interesting to ascertain in a future case where such a very large elimination of the substance may again be noted as in Bence Jones' and Magnus-Levy's cases, whether the normal globulins of the plasma are present, if at all, in normal amount. Supposing that the Bence Jones substance were actually produced in the manner indicated, it might be argued that as the plasma cells are normal components of the red marrow, traces, at least, of a corresponding body should even normally be demonstrable in the urine, which does not occur. But while accepting Wright's conclusion that the neoplasm, exclusive of its vessels and insignificant stroma, may have arisen from an abnormal proliferation of the plasma cells of the red marrow, it seems reasonable, I think, to assume that with the abnormal proliferation of the cells there is associated some deviation from their physiological function, of which, in turn, the appearance of the Bence Jones substance is the expression. According to this supposition, the body in question would appear in the urine, for the reason that it is foreign to the body, and we should accordingly also expect that following its artificial introduction into the blood current it would reappear in the urine. In the human being such experiments have, for obvious reasons, not been made, and it may be questionable whether the results of corresponding experiments in the case of animals can be directly adduced as evidence in the question under consideration. However, so far as they go they support the hypothesis that has been propounded. Kühne thus states that he sent a specimen of his material to Stokvis, and that the latter found the substance in the urine after intravenous injection as also following its introduction into the rectum.[1] Matthes also states that following the subcutaneous injection of the substance in dogs it reappears in the urine. Ellinger, on the other hand, reports that the intravenous injection even of large quantities (5 grammes in 75 c.c. of sodium carbonate solution injected into a dog weighing 6 killogrammes within two minutes) did not lead to the appearance in the urine of a substance that could be precipitated by salting with ammonium sulphate to saturation; but he adds that the filtrate of the salted urine gave a strong biuret reaction, which suggests that a substance or substances were present which were identical with or closely related to true peptone (in the sense of Kühne), and which may possibly have been derived from the injected material.

For the possible assumption that the Bence Jones substance originates during the process of gastric or intestinal digestion there is at present no basis.

NOTE.—Since writing the above an additional case of Bence Jones albuminuria (the twenty-third) has been observed in the service of Hijmans van den Bergh, in Rotterdam. An account of the clinical history and the post-mortem findings has not as yet appeared, but a detailed study of the patient's urine has been published by A. Gutterink and C. J. de Graff ("Ueber d. Darstellung einer krystallinischen Harnalbumose," *Zeit. f. Phys. Chem.*, 1902, vol. xxxiv. p. 392). Of special interest is the observation that the writers succeeded in crystallizing the albumin at will. A more detailed study of the chemical nature of the body is in progress.

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[1] Kühne. Ueber Hemialbumose im Harn, loc. cit., p. 214; and Stokvis, Maandblad voor Naturwetenschappen, 1872, No. 6.